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AN ALTERNATIVE SYSTEM FOR INCUBATION OF 'IN VITRO' PRIMARY IMMU--ETC(U)
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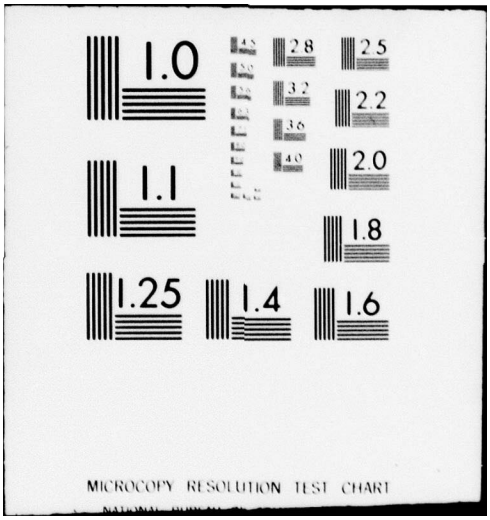
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In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on the Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

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## ABSTRACT

A system designed to provide a standard laboratory incubator with a reciprocating carriage for *in vitro* primary cultures is described. Data obtained with this system approximate results of the standard rocker system under most conditions and exceed results obtained by the rocker culture system in 24-well (16mm diameter well) plates at low cell concentrations.

## INTRODUCTION

Mishell and Dutton (1967) reported conditions for *in vitro* immunization of lymphocytes featuring a rocker platform requiring periodic feeding and gassing of cell cultures.

The alternative system of placing a moving platform within a laboratory sized $CO_2$ incubator has not been explored. This communication reports the development of such a device for incubation of spleen cell cultures and *in vitro* immunization.

## MATERIALS AND METHODS

### Mice

C57Bl/6 x DBA/2 ($B6D2F_1$) mice of either sex, 8-14 weeks of age, were purchased from Cumberland View Farms, Clinton, Tenn., U.S.A.

### Cell suspensions

Spleens from mice were teased into Hank's balanced salt solution (HBSS) without bicarbonate buffer and allowed to settle in 15-ml tubes on ice, for 5 min. Cell suspensions were decanted, counted and added to plates in varying numbers as noted in Results.

### Culture systems

Cells were cultured in Costar 24-well (16mm diameter well) plates. Each well received 0.5 ml of cell suspension. Varying numbers of cells and sheep red blood cells (SRBC) were used as noted in the Results. Mishell-Dutton media was used for cultures in all cases. Fetal bovine serum from Reheis Chemical Co. was tested for endotoxin and shown to be negative by the Limulus Amebocyte Lysate System (Microbiological Associates, Walkersville, MD, U.S.A.). Cell cultures were fed a nutritional cocktail consisting of 25 ml HBSS, 0.5ml

glutamine, 2 ml sodium bicarbonate (7.5%), 0.5 ml dextrose (50%), 1 ml essential amino acids and 0.5 ml nonessential amino acids. Cultures were incubated either on a rocker platform using Mishell-Dutton conditions or on the shifter device in a $CO_2$ incubator.

Plaque-forming cell assay

A modification of the hemolytic plaque assay of Jerne, Nordin, and Henry (1963) utilizing microscope slides was performed. Data given are for direct (IgM) plaque-forming cells (PFC) only.

Antigen

SRBC were a gift from Barbara Johnson of Dr. Donald E. Mosier's laboratory, National Institute of Allergy and Infectious Disea e, Bethesda, Md., U.S.A.

Description of the shifter system

The shifter power supply was fit onto a Hotpack, table-top $CO_2$ incubator, Model 351816 (Hotpack Corp., Philadelphia, Pa. U.S.A.). This incubator has forced air circulation and glass-wool insulated chamber walls. The power device for the shifter platform is a variable speed, two-channel Buchler peristaltic pump (Buchler Instruments, Ft. Lee, N.J., U.S.A.). The pump motor is attached to the incubator as illustrated in fig. 1. Briefly, the alterations made for this fit were: (1) removal of external pump assembly to expose the motor shaft; (2) fabrication of L-brackets to be pop-riveted to the motor case and screwed to the top surface of the incubator; (3) drilling of shaft holes through incubator chamber walls; and (4) mounting of brass bearings for the power transfer shaft.

A power transfer shaft with a cam on it was fabricated from Delrin stock (Reed Plastics, Rockville, Md., U.S.A.) and inserted through the

wall of the incubator. The cam was aligned with the push plate on the shifter carriage within the incubation chamber. Fig. 1 illustrates the shifter in place in the incubator.

The shifter carriage was fabricated from stock Plexiglas sheet. The moving portion of the carriage runs on four captive wheels. The stationary part of the carriage was anchored in the stainless steel shelving by means of pegs into drilled holes. Fig. 2 shows the shifter carriage with a microtiter plate carrier applied to it. Various carrier platforms were made to accommodate multi-well culture plates, culture on Petri-type dishes, or tubes of various sizes. These interchangeable carrier platforms are fitted to the shifter carriage by Plexiglas pegs.

The shifter was run at 15 cycles/minute while the rocker was maintained at 4 cycles/minute.

## RESULTS

Results are in the form of comparisons of PFC/well, while culture conditions are varied in 24-well Costar cluster plates incubated at 37°C either in a chamber on a Belco rocker or on the shifter device in a culture incubator.

### Spleen cell density

Concentration of cultured spleen cells varied from $1 \times 10^7$ - $3 \times 10^7$/ml cultured in 0.5-ml aliquots. PFC produced in primary *in vitro* immunization were comparable between the systems except for $1 \times 10^7$ cells/ml, fed or nonfed, where more PFC were observed from shifter than comparable rocker cultures. In some cases PFC from these conditions equaled the number of PFC from $2 \times 10^7$ cells/ml fed cultures on the rocker.

SRBC concentration

SRBC were added in a range extending from $1 \times 10^6$ - $3 \times 10^6$ cells/well. The optimal number of SRBC in culture ranged from $2 \times 10^6$ - $3 \times 10^6$ cells/well. The latter number often produced only nominal increases in PFC over $2 \times 10^6$ SRBC/well.

Culture feeding

Cultures were either not fed or fed 0.05 ml of nutritional cocktail. Cultures containing spleen cells at a $2 \times 10^7$/ml density or above benefited from feeding on either the rocker or shifter incubation systems. The data show that feeding cultures increased the number of plaques from the shifter system at low lymphocyte concentrations. Results of one experiment are summarized in table 1. Each data point is the mean of three values. Experiments altering these variables have been performed approximately 20 times giving qualitatively similar results.

## DISCUSSION

A basic comparability between data from shifter culture and rocker culture exists. This includes shifts of PFC numbers induced by altering numbers of spleen cells and SRBC. Furthermore, the similar requirements for feeding of cell cultures at high spleen cell concentrations are shown to exist in both systems. By way of general comparison, it would appear that the two systems react similarly to changes in these variables.

Specific data comparisons show that the shifter is superior to the rocker system in certain critical areas. The numbers of PFC at $1 \times 10^7$ spleen cells/ml from nonfed shifter cultures are comparable to the $2 \times 10^7$ spleen cells/ml cultures which are fed and incubated on the rocker. This suggests that feeding cultures is not as necessary on the shifter

(at low spleen cell concentrations) as it is on the rocker. A second comparison of $1 \times 10^7$ spleen cells/ml in fed shifter culture with $2 \times 10^7$ spleen cells/ml in fed rocker culture shows shifter data only slightly lower than that for rocker. This decrease is not in linear relationship to cell number and demonstrates the ability of the shifter culture system to enhance primary in vitro immunization at low cell numbers.

The advantages in the two comparisons made above are: an option is given in the former comparison as to the necessity of daily feeding of cultures; and in the second case, if one chooses daily culture feeding, half the number of cells per milliliter are required on the shifter than on the rocker to produce similar numbers of PFC.

No explanation is readily apparent for the advantages of shifter culture data made in these comparisons. Obviously the difference in plate motion could be a factor increasing cell interaction efficiency. However, it is probable that the relative constancy of heat, $CO_2$ partial pressure and humidity of the $CO_2$ culture incubator are major factors.

The final area in which the shifter and rocker culture systems may be compared is in size, cost and availability. The shifter is far smaller than any rocker and incubation box currently available and therefore very space saving. Furthermore, the shifter has no requirement for a walk-in incubator. This is an important factor in support of certain research projects. The cost is relatively modest and even when fabrication charges are applied, the cost should still be comparable to the rocker and incubator box. A forced air culture incubator is the only necessity for the shifter culture system.

REFERENCES

Jerne, N. K., A. A. Nordin and C. Henry, 1963, in: Cell-bound antibodies, eds, B. Amos and H. Koprowski (Wistar Institute Press, Philadelphia) p. 109.

Mishell, R. I. and R. W. Dutton, 1967, J. Exp. Med. 126, 423.

TABLE 1

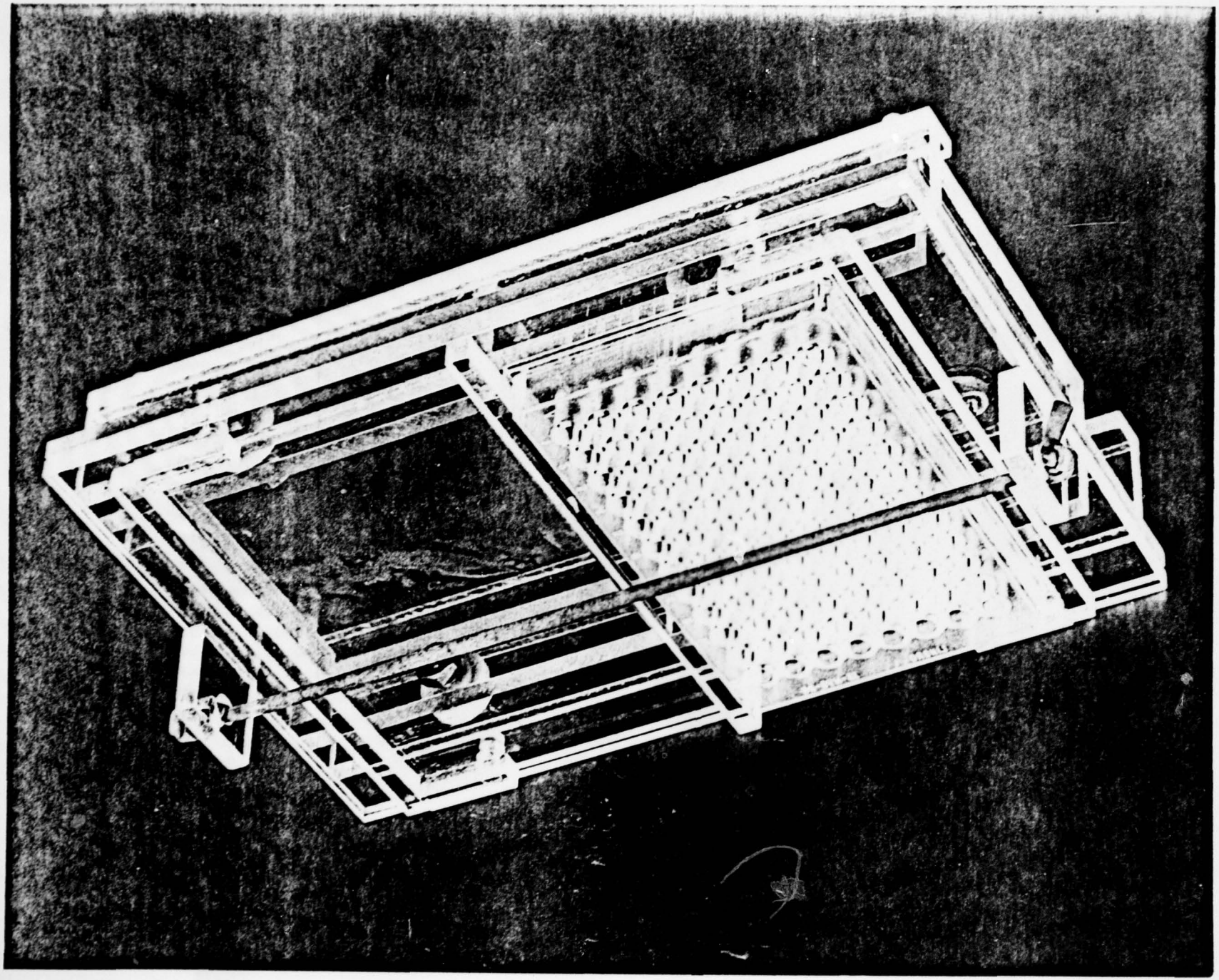
Numbers of plaque-forming cells from samples of equal numbers of cultured mouse spleen cells.

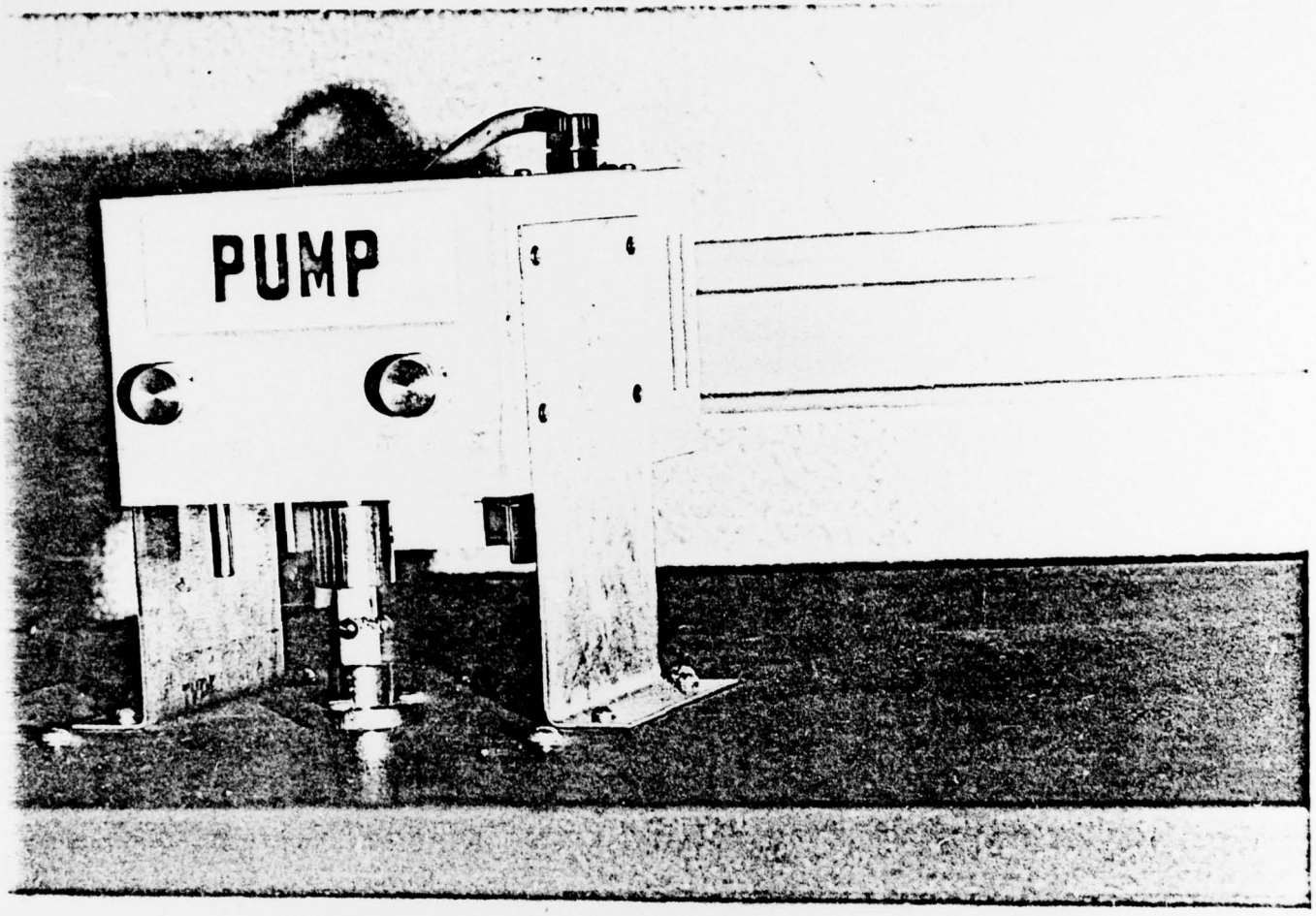
| Spleen cells/ml ($\times 10^7$) | Fed daily | PFC ($\times 10^6$) relative to SRBC/well | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Rocker | | | | Shifter | | | |
| | | 0 | 1 | 2 | 3 | 0 | 1 | 2 | 3 |
| 1 | + | 17 ± 2 | 198 ± 3 | 186 ± 2 | 137 ± 7 | 4 ± 1 | 171 ± 6 | 583 ± 14 | 601 ± 19 |
| | - | 1 ± 0 | 151 ± 2 | 128 ± 7 | 201 ± 2 | 10 ± 1 | 310 ± 13 | 260 ± 18 | 405 ± 7 |
| 2 | + | 4 ± 1 | 446 ± 30 | 368 ± 12 | 468 ± 17 | 22 ± 6 | 242 ± 14 | 252 ± 7 | 281 ± 9 |
| | - | 1 ± 0 | 8 ± 2 | 14 ± 1 | 8 ± 2 | 2 ± 1 | 67 ± 6 | 33 ± 3 | 50 ± 20 |
| 3 | + | 41 ± 3 | 509 ± 10 | 523 ± 6 | 526 ± 7 | 27 ± 1 | 297 ± 6 | 417 ± 7 | 482 ± 5 |
| | - | 4 ± 2 | 13 ± 1 | 7 ± 2 | 5 ± 1 | 4 ± 1 | 6 ± 1 | 11 ± 2 | 8 ± 1 |

LEGENDS FOR FIGURES

Fig. 1. Peristaltic pump attached by brackets to a convenient location on the incubator. Note shifter device connected to the power source by cammed shaft.

Fig. 2. Interchangeable carrier is shown fitted to the shifter carriage.

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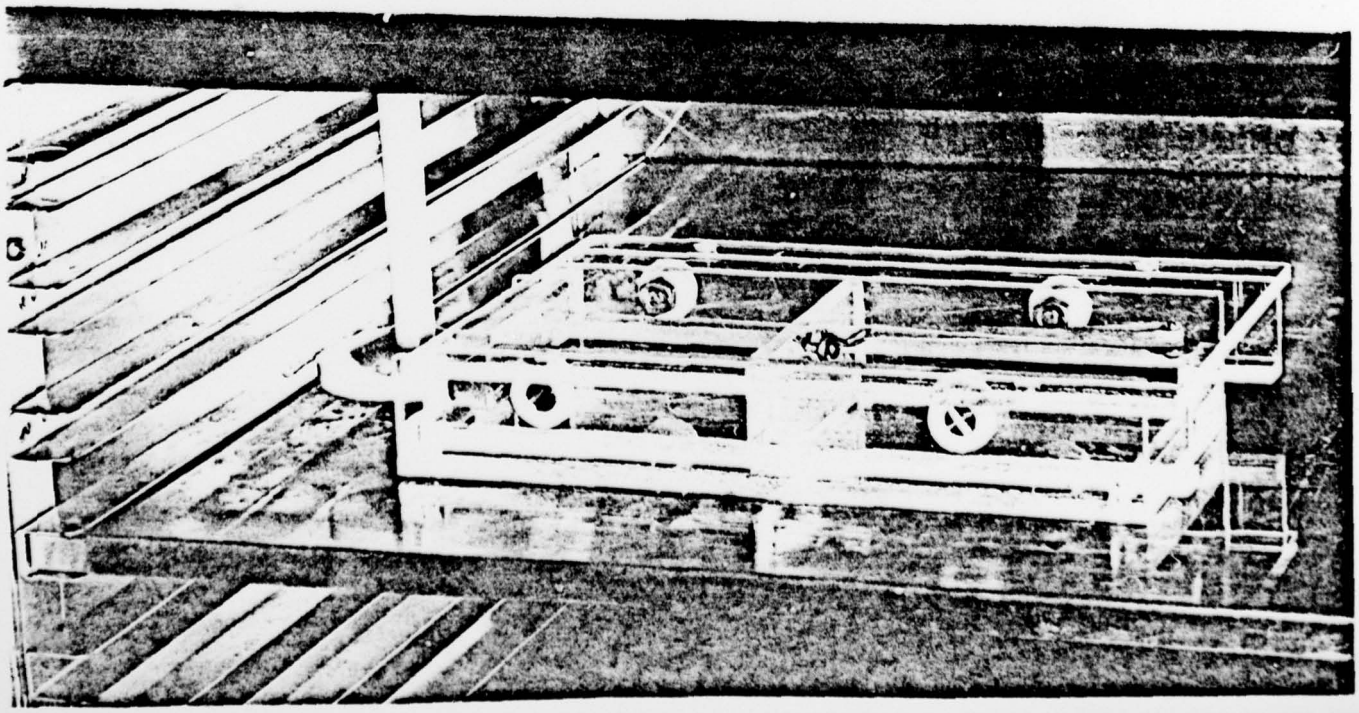

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